Computational modeling of macrophage polarization dynamics in skeletal malignancies. An integrated *in silico* and *in vivo* approach

Lo CH[1,2], Baratchart E[3], Basanta D[3] & Lynch CC[1]

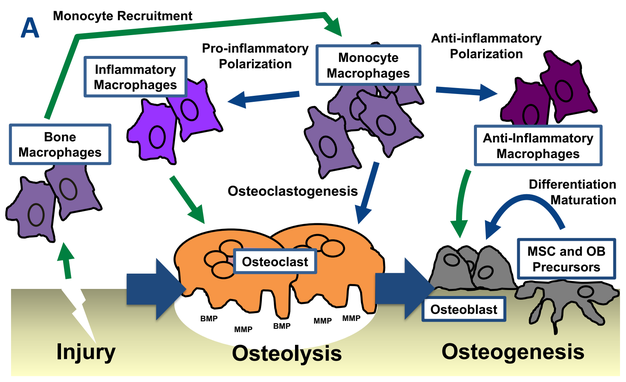
[1,3]Tumor Biology and Integrative Mathematical Oncology Depts., H. Lee Moffitt Cancer Center, 12902 USF Magnolia Drive, Tampa, FL

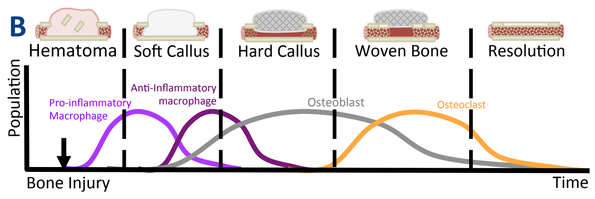
[2]Cancer Biology Ph.D. Program, University of South Florida, Tampa, FL

1. Bone-Metastatic Malignancies and The Vicious Cycle

- Various metastatic malignancies are osteophilic, including prostate cancer and breast cancer
- Most cancers are incurable at bone-metastatic stages and cause vicious cycle by disrupting osteolysis and osteogenesis, resulting in poorly-vascularized brittle bone with painful lesions susceptible to fractures
- Macrophage-targeted therapies have enjoyed success in some primary solid malignancies but their application in bone metastatic diseases are unknown
- Here we take an interdisciplinary *in silico*/*in vivo* approach to understand the largely unexplored role of macrophages in cancer-bone interaction

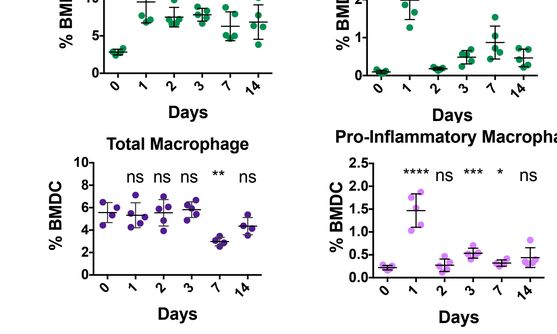
2. Macrophages in Bone Remodeling/Repair





A. Macrophage behavior in bone remodeling (Raggatt et al. 25285719, Schindeler et al. 18692584 Horwood 26498771). Arrows indicate **differentiation process** or **agonistic interaction**. **B**. Temporally-regulated polarized pro- and anti-inflammatory macrophages emerge to trigger clearance and repair responses bye osteoblasts and osteoclasts to restore the bone microenvironment.

3. Integrating Bench Top Experiments and Math Modeling



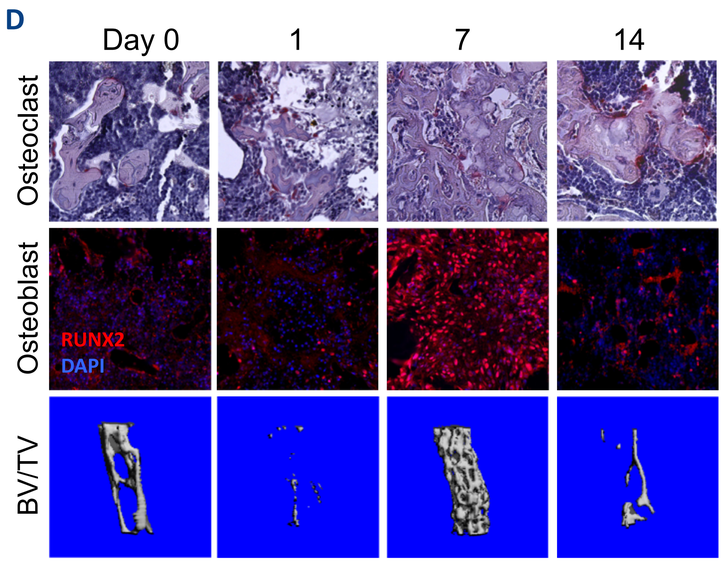
Math modeling facilitates the simultaneous observation of multiple cell populations and their interactions over time within a complex microenvironmental system. Math models powered by empirical and published parameters allow researchers to identify key components within networks of interactions, and make *in silico* predictions that can be validated biologically. **A**. The math model was initially parameterized to reflect cellular interactions underlying normal bone healing processes prior to the integration of metastatic prostate cancer cells. **B**. Building *in silico* models require a blend of empirical and published data to appropriately parameterize the model.

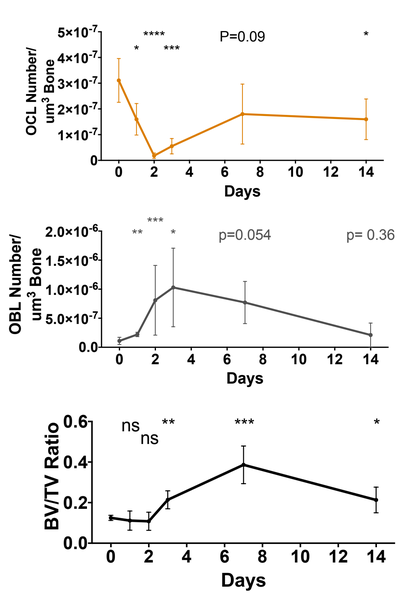
4. Macrophages Polarize into Pro- and Anti-Inflammatory States in Response to Bone Injury

A

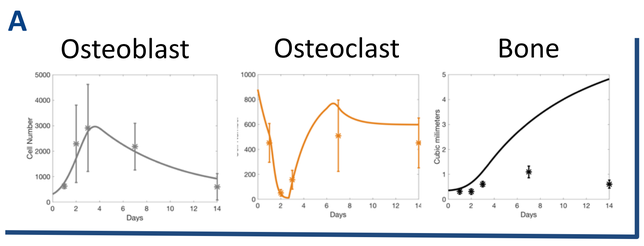
	Marker	Fluorophore
Phenotyping	CD11b	BV785
	Ly-6G	AF700
	Ly-6C	AF488
	F4/80	BUV395
Inflammatory Status	NOS2	APC
	ARG-1	PE
	TNF-a	BV650
	IL-4R	BV421
	MHC Class II	PE-Cy7
	Viability	NIR

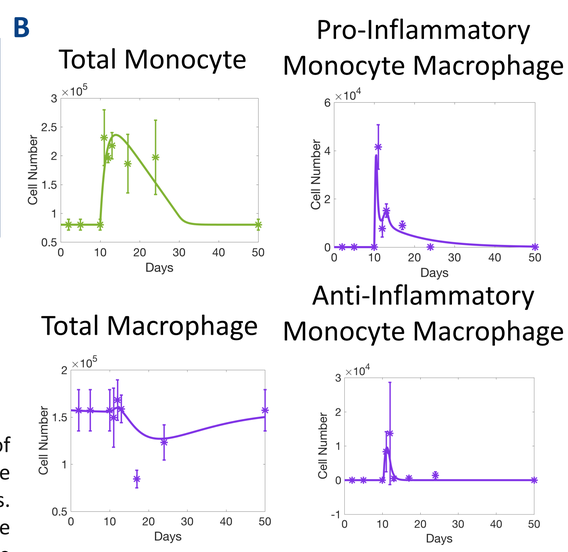
Bone injury was induced by performing mock intratibial injections on C57BL/6 mice (n=30). Sets of 5 mice were taken down for immunologic and histological analyses without injury to establish baseline quantitation and at Day 1, 2, 3, 7 and 14. Bone marrow was flushed from one tibia from each mice for assessing monocyte (CD11b+Ly6C+Ly6G-) macrophage (CD11b+Ly6C-Ly6G-) dynamics and the contralateral tibias were fixed in formalin for histology. **A.** Panel of marker antibodies were designed to phenotypically dissect myeloid subpopulations and their polarization states. **B.** Flow Cytometry analysis revealed dynamic shifts in monocyte and macrophage populations and polarization states in response to bone injury. **C.** Normalized quantitation revealed temporally distinct phases of monocytes and macrophages pro- (NOS2+) and anti-inflammatory (ARG1+) polarization, respectively. **D.** Fixed bones were subject to uCT analysis to reveal bone volume over time (BV/TV). Decalcified bones were sectioned and subject to osteoclast (TRAcP staining) and osteoblast (RUNX2 immunofluorescence) temporal quantitation.

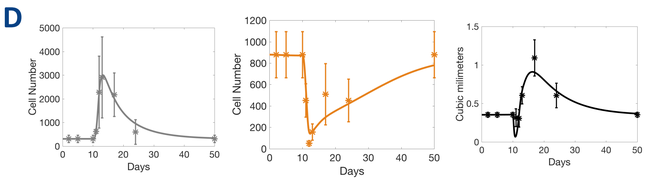




5. Monocyte Macrophage Polarization Controls Osteoclast and Osteoblast Activity during Bone Injury Repair







A. An Ordinary Differential Equation (ODE)-based model of osteoclast resorption and osteoblastic mineralization fails to capture bone dynamics by using known resorption and bone formation rates. **B.** An improved ODE model was generated to incorporate the function and dynamics of polarized monocyte macrophage populations to accurately recapitulate bone dynamics. **C.** The macrophage-based bone healing ODE model describes bone remodeling as a function of osteoclast, osteoblast dynamics and polarized macrophage dynamics. **D.** Modulation of osteoblast and osteoclast activity by polarized monocyte and macrophages were essential to recapitulate bone dynamics.

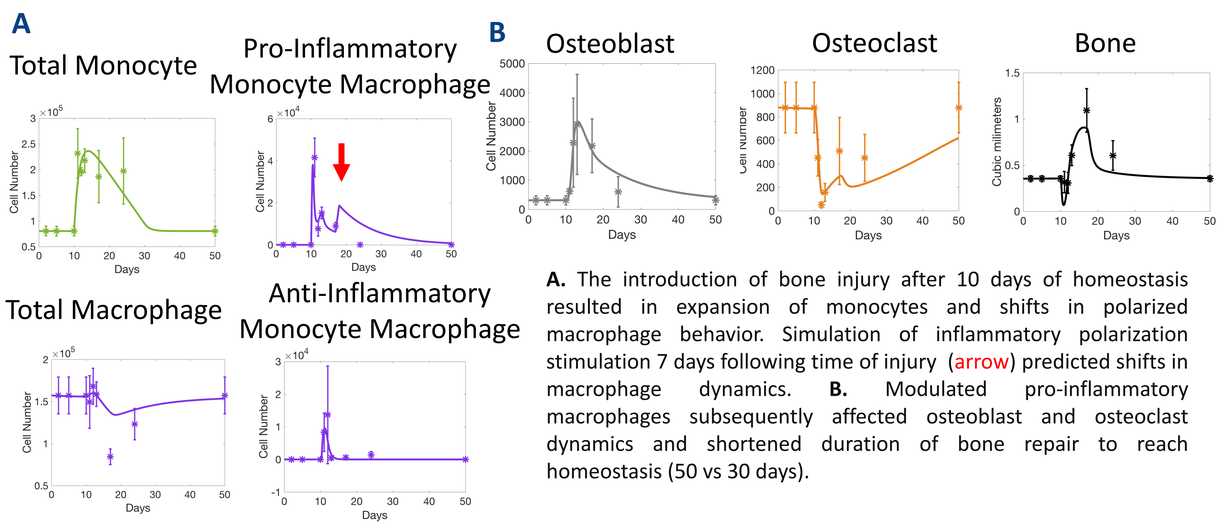
C

$$\frac{dOB}{dt} = \gamma_1 OCOB + \gamma_2 M2OB - \delta_{OB} BOB$$

$$\frac{dOC}{dt} = d_{OC} BMac - \delta_{OC} OBOC$$

$$\frac{dB}{dt} = -\delta_B(1+\alpha M1)OCL + \pi_B(1+\beta M2)OBL$$

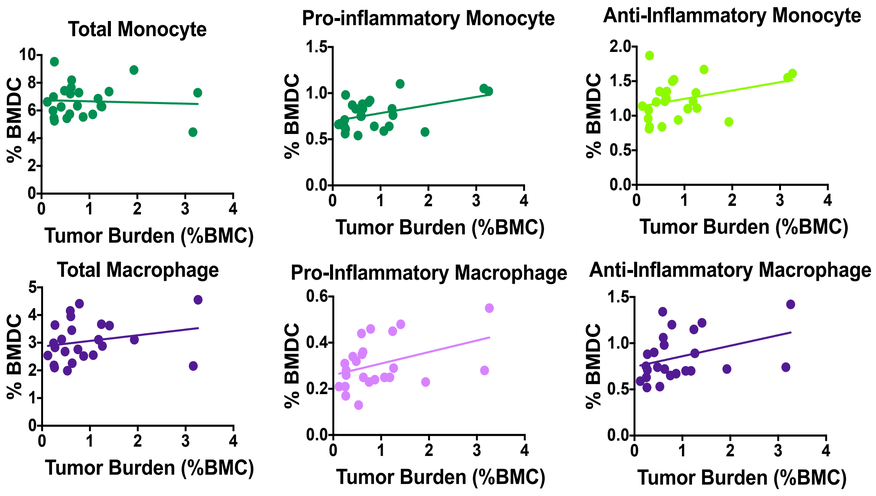
6. Modifying Macrophage Polarization Predicts Shorter Bone Healing Times



A. The introduction of bone injury after 10 days of homeostasis resulted in expansion of monocytes and shifts in polarized macrophage behavior. Simulation of inflammatory polarization stimulation 7 days following time of injury (arrow) predicted shifts in macrophage dynamics. **B.** Modulated pro-inflammatory macrophages subsequently affected osteoblast and osteoclast dynamics and shortened duration of bone repair to reach homeostasis (50 vs 30 days).

7. Multiple Myeloma Alters Monocyte and Macrophage Temporal Dynamics to Generate Osteolytic Disease

We are now incorporating macrophage dynamics into models of skeletal malignancy such as Multiple Myeloma. To this end, mice were inoculated with 5TGM1 multiple myeloma cells by tail vein injection (n=20; $1x10^6$ cells/mice) to establish bone lesions. Mice were sacrificed 7 days after injection and tibias were subjected to multimetric FACS analysis to assess tumor burden (IgG2b+) and corresponding macrophage polarization status. Linear regression curves reveal that tumor burden correlated with pro- and anti-inflammatory monocyte and macrophage polarization but not total populations.



8. Conclusions and Future Directions

- Macrophages are key players in the osteolytic and osteogenic response to bone injury
- The mathematical model, powered by empirical parameters, recapitulate the cellular dynamics of bone injury response
- *In vivo* injury model revealed persistent myeloid infiltration, and an initial expansion of pro-inflammatory, followed by, anti-inflammatory macrophages.
- Mathematical simulation of bone cell dynamics accurately reflected biological dynamics
- Math model can be manipulated to simulate existing therapeutics and offer insight into bone repair progression
- Bone-metastatic multiple myeloma generates pro- and anti-inflammatory macrophages over time *in vivo*
- ODE will be expanded into the cancer context for interrogating strategies to reduce cancer progression and bone pathology
- Rapid model predictions will assist in understanding tumor-bone biology and guide bench-top therapy design and testing

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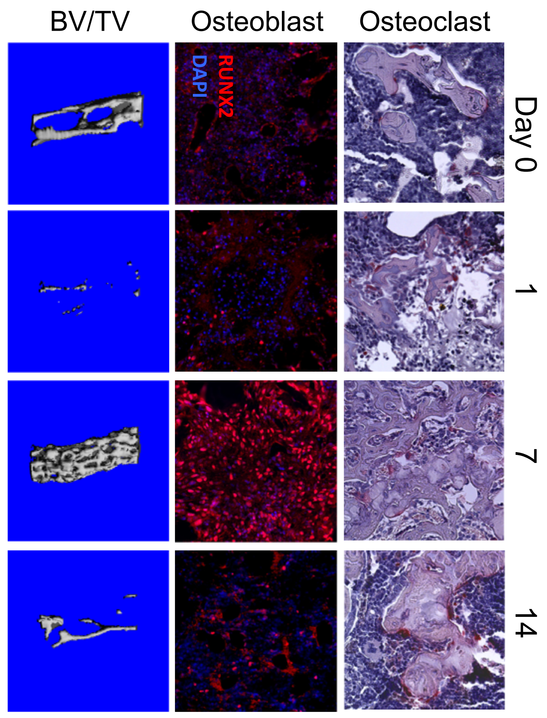
BV/TV
Osteoblast
Osteoclast
RUNX2
DAPI
Day 0
1
7
14